

### ***Amendments***

This listing of claims will replace all prior versions, and listings of claims in the application.

Claims 1-248. (Canceled).

249. (New) A method of producing a nucleic acid molecule comprising:

(a) providing a first nucleic acid molecule comprising at least a first promoter, a first positive selectable marker and at least a first recombination site;

(b) providing a second nucleic acid molecule comprising

(i) a second positive selectable marker, or portion thereof, which is not functional,

(ii) a negative selection marker, and

(iii) a second and a third recombination sites at least one of which will recombine with the first recombination site;

(c) forming a mixture *in vitro* between the first and second nucleic acid molecules and at least one site specific recombination protein, under conditions sufficient to cause recombination *in vitro* between the first and second nucleic acid molecules, thereby producing a third nucleic acid molecule in which the first promoter and the second positive selectable marker, or portion thereof, are operably linked to form a functional second positive selectable marker; and

(d) selecting for the nucleic acid molecules generated in step (c) which contain

(i) either the first or second positive selection marker, and

(ii) do not contain the negative selection marker.

250. (New) The method of claim 249, wherein the first nucleic acid molecule further comprises an origin of replication.

251. (New) The method of claim 249, wherein the second nucleic acid molecule further comprises one or more functional antibiotic resistance genes.

252. (New) The method of claim 249, wherein the negative selection marker is a toxic gene.

253. (New) The method of claim 252, wherein the toxic gene is a restriction enzyme.

254. (New) The method of claim 253, wherein the restriction enzyme is *DpnI*.

255. (New) The method of claim 249, wherein the first positive selectable marker is an antibiotic resistance gene.

256. (New) The method of claim 249, wherein the second positive selectable marker, or portion thereof, is an antibiotic resistance gene selected from the group consisting of a chloramphenicol resistance gene, or a portion thereof; an ampicillin resistance gene, or a portion thereof; a methicillin resistance gene, or a portion thereof; a tetracycline resistance gene, or a portion thereof and a kanamycin resistance gene, or a portion thereof.

257. (New) The method of claim 249, wherein the second positive selectable marker, or portion thereof, is a chloramphenicol resistance gene, or a portion thereof.

258. (New) The method of claim 249, wherein the first and second and third recombination sites are selected from the group consisting of *lox* sites, *att* sites, and mutants thereof.

259. (New) The method of claim 249, wherein the first and second and third recombination sites are selected from the group consisting of *lox* sites and *att* sites.

260. (New) The method of claim 249, wherein the first and second and third recombination sites are *lox* sites.

261. (New) The method of claim 260, wherein the *lox* sites are *loxP* sites.

262. (New) The method of claim 249, wherein the first and second recombination sites are *att* sites.

263. (New) The method of claim 262, wherein the *att* sites are selected from the group consisting of *attB* sites, *attP* sites, *attL* sites and *attR* sites.

264. (New) The method of claim 249, wherein the first promoter is located immediately adjacent to the first recombination site.

265. (New) The method of claim 249, wherein the second positive selectable marker, or portion thereof, is located immediately adjacent to the second recombination site.

266. (New) The method of claim 249, wherein the at least one site-specific recombination protein is selected from the group consisting of Cre, Int, IHF, Xis, FLP,  $\gamma\delta$ , Tn3 resolvase, Hin, Gin, Cin and combinations thereof.

267. (New) The method of claim 249, wherein the at least one site-specific recombination protein is Cre.

268. (New) The method of claim 249, wherein the at least one site-specific recombination protein is selected from the group consisting of Int, IHF and Xis.

269. (New) The method of claim 249, wherein the first nucleic acid molecule or the second nucleic acid molecule or the third nucleic acid molecule is a vector.

270. (New) The method of claim 269, wherein the vector is an expression vector.

271. (New) The method of claim 249, wherein the first nucleic acid molecule or the second nucleic acid molecule is linear.

272. (New) The method of claim 249, further comprising contacting at least one host cell with the mixture, and selecting for a host cell comprising the third nucleic acid molecule.

273. (New) The method of claim 272, further comprising selecting against a host cell comprising the first or the second nucleic acid molecule.

274. (New) The method of claim 272, further comprising selecting against a host cell comprising the first and the second nucleic acid molecule.

275. (New) The method of claim 272, wherein the host cell is a prokaryotic cell.

276. (New) The method of claim 272, wherein the host cell is a bacterial cell.

277. (New) The method of claim 272, wherein the host cell is an *Escherichia coli* cell.

278. (New) The method of claim 249, further comprising introducing the third nucleic acid molecule into a host cell.

279. (New) The method of claim 249, further comprising introducing the third nucleic acid molecule into a host cell and expressing the second positive selectable marker, or portion thereof.

280. (New) The method of claim 279, wherein the host cell is an *Escherichia coli* cell.